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COMMUNICATION

Manufacturing of anisotropic particles by site specific oxidation of thiols†

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A novel method for the manufacturing of functional anisotropic particles based on an inexpensive and straightforward electrochemical approach is presented. The method enables large-scale manufacturing of anisotropic particles as well as fabrication of multifunctional beads which may be used in the design of barcodes for multiplex diagnostics.

Micro-bead based technologies are currently widely employed in diverse disciplines of chemistry and biology. In most applications the utilised beads are isotropic or symmetric, but recently, anisotropic beads (e.g. Janus-, patchy- or multicompartiment particles) have been gaining increasing interest.¹ Such anisotropic beads are usually heterogeneous with respect to their chemical or physical properties like magnetisation,² surface functionality³ or morphology.⁴ There are several published concepts addressing the manufacturing of anisotropic beads, based on microcontact printing,⁵ microfluidic synthesis,⁶ beads-on-beads,⁷ sonochemical synthesis⁸ and lithography.⁹ The multi-functionality of the anisotropic beads has made them interesting tools for the design of barcodes for multiplex diagnostics.¹⁰ However, as only a few of these methods allow the manufacturing of multiple patched anisotropic beads there is a need for the development of new undemanding and up-scalable techniques for this purpose.

This communication describes for the first time an inexpensive and straightforward electrochemical approach for the selective and partial functionalisation of magnetic non-conducting polymer micro-beads yielding anisotropic particles. The method is, in short, based on an electrochemical site specific oxidation of small segments on the thiolated particle surface. This site specific oxidation results from the fact that the thiolated particles in contact with an electrode surface are oxidised only at the particle–electrode interface, as is depicted in Fig. 1. In a previous publication it was demonstrated that an AFM tip or an alumina stamp could be used as an electrode to obtain electrochemical-contact printing of flat thiolated conducting

surfaces.¹¹ In that case, the thiolated surface was thus used as one of the electrodes. In the present case, the oxidation involves the segments of the thiolated non-conducting polymer beads in contact with the electrode. In both approaches, the oxidation of the thiols generates reactive thiolsulfonates and thiolsulfonates which subsequently can be used to functionalise the particles. The new approach and its application with respect to the manufacturing of multiple partially

Fig. 1 A schematic illustration of the electrochemical process employed to generate partially oxidised bead surfaces. (a) Magnetic bead from bulk solution attracted to the electrode/solution interface by a permanent magnet. (b) Oxidation of the thiols in contact with the glassy carbon working electrode yielding spots of reactive thiolsulfonates and thiolsulfonates.

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functionalised particles is unique since it enables repeatable site specific oxidation of non-conducting particles and as it provides new exciting possibilities for straightforward large-scale manufacturing of anisotropic particles.

The general process to fabricate and characterize the anisotropic particles is summarised below (a more detailed description can be found in the ESI†). First, particles are functionalized with thiols as follows. Amino-conjugated magnetic latex beads (Micromer® –M PEG-NH₂), with a diameter of 5 μ m, were reacted with *N*-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) followed by reaction with dithiothreitol (DTT) (see Scheme S1†) to yield approximately 10⁸ thiol groups on each particle. The thiolated beads, suspended in a solution of 10 mM phosphate buffered saline (PBS), were electrochemically oxidised in a three-electrode set-up comprising a glassy carbon or gold working electrode, a gold counter electrode and either a Ag/AgCl or a platinum quasi reference electrode (see ESI†). Beads from the bulk solution were attracted to the working electrode/solution interface by a permanent magnet mounted underneath the working electrode, as illustrated in Fig. 1a. A potential of +1.0 to +2.31 V vs. Ag/AgCl was then applied to the working electrode for 0.1 to 60 seconds. This resulted in an oxidation of the thiol layer of the beads present at the working electrode/solution interface. It has previously been shown that surface bound thiols on a conducting planar substrate can be oxidised to reactive thiolsulfonates (–SO) and thiolsulfonates (–SO₂).¹² The formation of thiolsulfonates and thiolsulfonates upon the oxidation of thiols has likewise previously been verified using electrochemistry coupled to electrospray mass spectrometry.¹³ However, in the present case, only the thiols sufficiently close to the electrode were oxidised to reactive thiolsulfonates and thiolsulfonates as schematically depicted in Fig. 1b. This straightforward approach can consequently be used for selective and partial electrochemical oxidation of electrically non-conducting beads. For sufficiently conducting particles, a homogeneous oxidation of the thiolated surface can, on the other hand, be obtained.

To verify the formation of spots of thiolsulfonates and thiolsulfonates on the beads, experiments were also carried out with a flat thiolated model surface which was electrochemically oxidised at +2.31 V vs. Ag/AgCl for 60 seconds using a glassy carbon working electrode. In these experiments, a piece of a silicon wafer, silanised with (3-mercaptopropyl) methyl dimethoxy silane (MPMDMS) (see Scheme S2†), was used as the flat thiolated surface, placed directly on the glassy carbon electrode. This surface was then investigated using X-ray Photoelectron Spectroscopy (XPS), employing a Scienta ESCA-300 spectrometer and monochromatic Al-K _{α} radiation with a photon energy of 1487 eV. The sulfur 2s photoelectron spectra which were recorded before and after electrochemical oxidation of the thiolated Si surface are shown in Fig. 2. While the peak at 228 eV binding energy (BE) present in both Fig. 2a and b originates from surface thiols, the broad peak at higher binding energies, seen only after oxidation, is typical for oxidised thiols, e.g. thiolsulfonates (233 eV BE) and thiolsulfonates (231 eV BE).¹¹ Based on the peak intensities in the S2s spectra, it can be estimated that a major part of the surface thiols were oxidised mainly to thiolsulfonates when using a potential of +2.31 V vs. Ag/AgCl.

Finally the electrochemically oxidised areas were functionalised with immunoglobulin G (IgG) molecules to obtain the protein anisotropic particle surfaces (see Scheme S3†). Prior to the reaction with the beads, IgG was treated with fluorescein isothiocyanate (FITC) and thereafter thiolated in the same manner as the beads. The

Fig. 2 Sulfur 2s photoelectron spectra for a thiolated silicon surface (a) prior to and (b) after electrochemical oxidation at the interface of a glassy carbon working electrode using +2.31 V vs. Ag/AgCl for 60 s.

extent of the conjugation of the thiolated IgG(FITC) to the oxidised regions on the beads was then evaluated employing a fluorescence microscope equipped with filters providing an excitation wavelength of 494 nm and an emission wavelength of 520 nm. Fig. 3a depicts fluorescence microscope images of beads, exhibiting one functionalised area appearing as a fluorescent spot. The latter beads were oxidised using the glassy carbon electrode employing a potential of +1.61 V vs. Ag/AgCl. At more positive oxidative potentials, e.g. up to +2.31 V vs. Ag/AgCl, functionalised areas were not obtained on the bead surfaces. In these cases there were thus no reaction with IgG (FITC)-SH, most likely, due to an overoxidation of the surface thiols (probably yielding sulfates), and/or a degradation of the beads surface itself. When thiolated beads were electrochemically oxidised using a gold working electrode, rather than a glassy carbon electrode, both partial- and weak homogeneous oxidation of the whole bead surfaces could be obtained. The homogeneous oxidation can be explained by the simultaneous generation of oxidising Au(III) chloride complexes in accordance with previous reports.¹⁴

The experimental results clearly show that the present approach allows the generation of partial and selective functionalisation of non-conducting beads. Preliminary results also show that it is possible to generate more than one partially functionalised area on each bead, resulting in a patchy-like particle. By including several potential steps and by subsequently releasing and attracting the beads to the electrode/solution interface, new segments of the particle may then be oxidised (see Fig. S1†). To study this concept, thiolated beads were electrochemically oxidised on a gold working electrode utilizing a four-cycle procedure prior to the reaction with IgG(FITC)-SH. As depicted in Fig. 3b, this generated a mixture of beads functionalised with up to four visible functionalised areas. Further work to optimise this process to enable a repeatable oxidation of a monolayer of particles yielding particles with a predefined number of functionalised areas is in progress. Such a monolayer based process would also make subsequent separations of unreacted and reacted particles redundant. Compared to the beads electrochemically oxidised with

Fig. 3 Fluorescence microscope images of partially functionalised beads showing (a) beads oxidised with a glassy carbon working electrode with one potential pulse (+1.61 V vs. Ag/AgCl for 60 s) generating one functionalised area and (b) beads oxidised with four potential pulses (+1.0 V vs. Ag/AgCl for 0.1 s) using a gold working electrode resulting in beads with (i) one, (ii) two, (iii) three and (iv) four visible functionalised areas. The fluorescent dots were obtained as a result of the IgG(FITC) thiols reacting with the thiolsulfonates and thiolsulfonates in the partially oxidised areas. The length of scale bars is 5 μm .

the glassy carbon electrode, the functionalised areas were somewhat larger when the gold working electrode was used. The spots obtained after oxidation on the glassy carbon and on the gold working electrodes exhibited an area of $0.47 \pm 0.05 \mu\text{m}^2$ and $2.03 \pm 1.01 \mu\text{m}^2$, respectively, as evaluated with an image processing and analysing program (see ESI†). Since the particles were 5 μm in diameter, corresponding to a total surface area of $19.6 \mu\text{m}^2$, approximately 2 and 10% of the particle surface, respectively, were thus oxidised and functionalised. The larger spots, which also varied more in size, were characterised with XPS to contain Au(I) species (see ESI†). The latter is not surprising as the formation of gold(I)-thiolates from Au(III) chloride in the presence of thiols is well known and has been utilised for formation of thiol-supported gold nanoparticles.¹⁵ With the gold electrode, the conjugation of the thiolated IgG(FITC) to the beads therefore most likely takes place *via* an interaction between the thiols in the biomolecule and the formed Au(I)-S complex on the beads. The latter are formed as a result of an oxidation of the thiols by the released Au(III) rather than *via* a direct oxidation on the electrode as proposed when using the glassy carbon working electrode which explains both the larger areas of the spots as well as the possibility to obtain homogeneous oxidation with the gold electrode.

Conclusions

A straightforward technique for the generation of anisotropic particles based on electrochemical segmental oxidation of non-conducting thiolated magnetic beads has been demonstrated. This approach can be used both in the form of a parallel process for partial immobilisation of different kinds of molecules on beads (which could be useful as a tool for bar-coding of beads), as well as for the fabrication of multi-functionalised beads. Further work to generate and optimise the shapes of multi-functionalised areas on the surfaces of beads is currently in progress.

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